TITLE: Vascular responses of the extremities to transdermal application of vasoactive agents in Caucasian and African descent individuals

AUTHORS: Matthew J. Maley, James R. House, Michael J. Tipton and Clare M. Eglin

ADDRESS FOR CORRESPONDENCE:
Matthew J. Maley
Extreme Environments Laboratory
Department of Sport and Exercise Science
University of Portsmouth
Portsmouth, PO1 2ER, UK
Email: matthew.maley@port.ac.uk
Telephone: +44 23 9284 6395

ABSTRACT

Purpose

Individuals of African descent (AFD) are more susceptible to non-freezing cold injury than Caucasians (CAU) which may be due, in part, to differences in the control of skin blood flow. We investigated the skin blood flow responses to transdermal application of vasoactive agents.

Methods

Twenty four young males (12 CAU and 12 AFD) undertook three tests in which iontophoresis was used to apply acetylcholine (ACh 1 w/v %), sodium nitroprusside (SNP 0.01 w/v %) and noradrenaline (NA 0.5 mM) to the skin. The skin sites tested were: volar forearm, non-glabrous finger and toe, and glabrous finger (pad) and toe (pad).

Results

In response to SNP on the forearm, AFD had less vasodilatation for a given current application than CAU (P = 0.027 to 0.004). ACh evoked less vasodilatation in AFD for a given application current in the non-glabrous finger and toe compared with CAU (P = 0.043 to 0.014) with a lower maximum vasodilatation in the non-glabrous finger (Median [interquartile], AFD n=11, 41[234]%, CAU n=12, 351[451]%, P = 0.011) and non-glabrous toe (Median [interquartile], AFD n=9, 116[318]%, CAU n=12, 484[720]%, P = 0.018). ACh and SNP did not elicit vasodilatation in the glabrous skin sites of either group. There were no ethnic differences in response to NA.

Conclusion

AFD have an attenuated endothelium-dependent vasodilatation in non-glabrous sites of the fingers and toes compared with CAU. This may contribute to lower skin temperature following cold exposure and the increased risk of cold injuries experienced by AFD.

KEY WORDS: ethnicity; vasodilatation; vasoconstriction; glabrous; non-glabrous

ABBREVIATIONS

ACh	Acetylcholine
AFD	African descent
AVA	Arteriovenous anastomoses
CAU	Caucasian
ED50	Half-maximal effective dose
IQR	Interquartile range
MAP	Mean arterial pressure
Mdn	Median
NA	Noradrenaline
NFCI	Non-freezing cold injury
SNP	Sodium nitroprusside

INTRODUCTION

The feet and hands of individuals who experience local cold tissue temperatures (0 °C to 20 °C) for prolonged periods are at risk of non-freezing cold injury (NFCI) (Ungley and Blackwood 1942). Although the feet are at the greatest risk of suffering a NFCI, various peripheral regions including hand, face and ears are also susceptible (Ungley et al. 1945). Daanen and van der Struijs (2005) showed that out of 57 individuals who suffered a NFCI during winter military operations, 72 % occurred in the feet, 25 % in the hands with the remaining injuries occurring on the head. In the UK military, the proportion of patients seen with NFCI of the hands was 41 % in 2007 seen by the Institute of Naval Medicine's Cold Injury Clinic (Oakley 2009). Symptoms of this injury may last for many years and often include pain, numbness and hyperhidrosis which, combined with cold hypersensitivity of the injured limb, can lead to increased susceptibility to further cold injuries (Ungley et al. 1945; Golden et al. 2013). This type of injury is a concern for those involved in fishery and agricultural work, military operations as well as those participating recreational activities in the cold (e.g. skiing, mountaineering) (Hashmi et al. 1998; Mäkinen et al. 2009; Russell et al. 2013). Additionally, NFCI has come close to deciding the outcome of military conflicts (Golden et al. 2013) and has had financial implications in the form of occupational claims for compensation.

There are various predisposing factors for cold injuries, these include: high altitude (Hashmi et al. 1998); age (> 62 years) (Koutsavlis and Kosatsky 2003; Sawada 2005); gender (females) (Army Medical Surveillance Activity 2013), nicotine (Cleophas et al. 1982; Waeber et al. 1984) and caffeine consumption (Kim et al. 2013). Ethnicity is also a risk factor; individuals of black African descent (AFD) are more susceptible than Caucasian (CAU) individuals to NFCI (Miller and Bjornson 1962; Taylor 1992; Candler and Ivey 1997; Conway and Husberg 1999; DeGroot et al. 2003; Burgess and Macfarlane 2009). It is thought that sustained skin blood flow in the extremities in low environmental temperatures can prevent local cold injuries such as NFCI (Lewis 1930; Wilson

and Goldman 1970; Daanen and van der Struijs 2005). We have previously observed that during hand immersion in cold water (8 °C) for 30 minutes and subsequent rewarming of dry skin in 30 °C air, AFD experience greater vasoconstriction and also rewarm later and more slowly than CAU (Maley et al. 2014). Furthermore, during hand and forearm cooling and rewarming the onset of finger vasoconstriction and vasodilatation occurs at a warmer skin temperature in AFD compared with CAU, resulting in a greater 'dose of cold' (Maley et al. 2014).

Taken together, it appears that the greater susceptibility of AFD to NFCI may be due to differences in the control of skin blood flow between these two ethnic groups. It is important to investigate both glabrous and non-glabrous extremity skin sites as NFCI occurs at both sites, but the control of skin blood flow appears to differ between these sites. The control of skin blood flow is intricate. Sympathetic vasoconstrictor and vasodilator nerves innervate all areas of non-glabrous (hairy) skin, whereas glabrous (hairless) skin is thought to be innervated solely by vasoconstrictor nerves (Sarnoff and Simeone 1947; Johnson et al. 1995) although this has been contested (Lundberg et al. 1989). An important feature of the control of skin blood flow in glabrous skin is the existence of arteriovenous anastomoses (AVA), which are thick muscular, low resistance vessels that allow high flow rates directly from arterioles to venules (Grant 1930; Grant and Bland 1931; Clark 1938). Glabrous regions, such as the finger tips, have up to 236 AVA per cm^2, whereas no AVA are found in non-glabrous regions of the hand or foot, or the volar surface of the forearm (Grant and Bland 1931).

The control of skin blood flow has been examined using agents that influence endothelium-dependent or independent vasodilatation, as well as vasoconstriction. Previous investigations have reported that AFD experience an attenuated response to endothelium-dependent vasodilators (e.g. methacholine or acetylcholine) compared with CAU in the forearm circulation (Stein et al. 1997; Jones et al. 1999) although this was not supported by Kahn *et al.* (2002).

Blood flow responses to an endothelium-independent vasodilator (e.g. sodium nitroprusside or glyceryltrinitrate) appears to be mixed with some observations showing similar responses between AFD and CAU (Kahn et al. 2002; Melikian et al. 2007), whilst others have shown a lower blood flow response in AFD compared with CAU (Stein et al. 1997; Cardillo et al. 1999). The vascular responses to vasoconstrictor agents (e.g. angiotensin II or phenylephrine) has not been as extensively studied, but responses may (Stein et al. 2000), or may not (Jones et al. 1999), differ between ethnic groups.

The skin blood flow responses to local application of vasoactive agents in CAU and AFD in skin sites susceptible to NFCI are not known. Therefore, the aim of the present study was to examine endothelium-dependent and independent dilatation by using acetylcholine (ACh) and sodium nitroprusside (SNP), respectively. We also investigated any potential vasoconstrictor differences by utilising noradrenaline (NA). Both non-glabrous and glabrous skin sites on the fingers and toes as well as the forearm were tested. It was hypothesised that AFD would experience an attenuated response to ACh and SNP and an exaggerated response to NA compared with CAU.

METHODS

Participants

12 CAU and 12 AFD male volunteers participated in the study. All participants were non-smokers, were free from any vascular or blood disorders including hypertension, sickle cell disease, diabetes and Raynaud's phenomenon, with no history of either freezing or non-freezing cold injuries. Participants' history of cold exposure was ascertained by questionnaire with each ethnic group reporting similar exposure to cold. Ethnicity was determined by self-classification and all participants were UK residents at the time of testing. All CAU were born in the UK. Four AFD participants were born in the UK whilst eight had resided in the UK for an average of six years; four were born in

Zimbabwe, two were born in Nigeria, one was born in Ghana and one was born in Italy. Prior to testing, participants were asked to refrain from consuming alcohol for 24 hours and participating in exercise or consuming caffeine for 12 hours.

Experimental procedures and measurements

Participants attended the laboratory on three occasions separated by at least 24 hours where they received local transdermal application of either ACh, SNP or NA using iontophoresis in a balanced order. The technique of iontophoresis has been described previously (Morris and Shore 1996; Roustit et al. 2014). Briefly, iontophoresis is a non-invasive method of transdermal drug delivery which transfers charged molecules using a low-intensity electric current into and through the skin to a depth of approximately 2 to 4 mm (Anderson et al. 2003).

All experiments were carried out in a quiet temperature controlled chamber. Environmental (dry bulb) temperature was maintained at 23 °C for ACh and SNP protocols. The NA protocol was conducted at a (dry bulb) temperature of 24 °C. Pilot testing demonstrated that participants at rest in these environmental temperatures were within the “vasomotor zone” (i.e. neither fully vasoconstricted nor vasodilated) and provided the ideal baseline to observe vasodilatation and vasoconstriction respectively. Each participant was supine throughout the experiment. Blood pressure, from the contralateral arm used for iontophoresis, was recorded pre and post each application of iontophoresis and measured using an automated monitor (Minimon 7137 Plus, Kontron Instruments, UK). All participants rested for 20 minutes in a supine position to allow skin temperature and skin blood flow to stabilise before application of iontophoresis.

Iontophoresis was applied to the left side of the body to the volar aspect of the forearm, non-glabrous region of the middle finger, non-glabrous region of the Great toe, glabrous region of the middle finger pad and the glabrous region of

the Great toe pad. The order of sites tested was balanced. Each skin site was cleaned with deionised water prior to iontophoresis.

In preliminary studies we observed that iontophoresis was difficult to conduct on AFD as it appeared that they had greater skin resistance which limited the current that could be applied. Approximately 25 μA could be consistently delivered to both AFD and CAU participants. We therefore adapted our protocol based on previous investigations using intermittent pulses of similar duration (Morris and Shore 1996; Hendry and Marshall 2004; Easter and Marshall 2005).

Iontophoresis was performed using both an anode and cathode connected to a battery powered iontophoresis controller (MIC2, Moor Instruments, UK). The iontophoresis chamber, which is a small Perspex ring (MIC-ION1R-P1, Moor Instruments, UK) with an inner diameter of 8 mm, was filled with approximately 0.2 mL of the relevant drug solution. A laser Doppler probe (VP1T / 7, Moor Instruments, UK), utilised to measure skin temperature and skin blood flow, was placed into the Perspex ring and connected to a laser Doppler flowmetry monitor (moorVMS-LDF, Moor Instruments, UK). Laser Doppler and iontophoresis data were recorded using a data acquisition system and software (Powerlab and LabChart 7, AD Instruments, New Zealand).

Protocols

Acetylcholine (ACh)

ACh was used at the anode with the cathode placed proximally to the site of interest. The protocol consisted of six pulses of 25 μA followed by one pulse of 50 μA and one of 100 μA for 20 seconds all separated by 60 second intervals in which no current was applied. After an interval of five minutes the protocol was repeated on the next skin site.

Sodium nitroprusside (SNP)

SNP was used at the cathode with the anode placed proximally to the site of interest. The protocol consisted of six pulses of 25 μA followed by one pulse of 50 μA and one of 100 μA for 20 seconds all separated by 120 second intervals in which no current was applied as the dilator response to SNP takes longer to develop than ACh (Ramsay et al. 2002; Hendry and Marshall 2004). After an interval of five minutes the protocol was repeated on the next skin site.

Noradrenaline (NA)

NA was used at the anode with the cathode placed proximally to the site of interest. The protocol consisted of six pulses of 25 μA followed by one pulse of 50 μA and one of 100 μA for 30 seconds all separated by 60 second intervals in which no current was applied. After an interval of five minutes the protocol was repeated on the next skin site.

Drugs

ACh was obtained as Miochol-E (Bausch & Lomb, Surrey, UK) and prepared immediately prior to use to a concentration of 1 w/v %. SNP (Rottapharm Madaus, Barcelona, Spain) was dissolved into water for injection to a concentration of 0.01 w/v %. NA (Hospira, Leamington Spa, UK) was diluted into water for injection to a concentration of 0.5 mM. As SNP and NA are photosensitive, all solutions were wrapped in foil and stored in the dark prior to use, with stock solutions being used within eight hours.

Pilot studies using the vehicle for each drug on the skin sites used for the main protocol showed that the only skin site to show an increase in skin blood flow was the forearm when the cathode was used (for SNP only); this is addressed within the discussion section.

Data analyses

Due to high skin resistance it was not possible to deliver all of the current pulses in each skin site for all participants; this occurred more in the AFD participants. As a consequence the number of participants contributing to the mean data decreased as the cumulative current increased (see Fig. 1 and 2). Participants who were able to receive the iontophoresis charge were included in the data analysis. Analysis of the skin blood flow data showed the results were similar if only those who completed the protocol were included compared to inclusion of all participants until they were not able to receive the desired current. Therefore, as we wanted to include as many participants as possible in the analysis we included all participants until they were not able to receive the desired current. As blood pressure remained constant throughout the study (see Table 1), skin blood flow is expressed in laser Doppler units rather than cutaneous vascular conductance (flux/mean arterial pressure). Responses evoked in the cutaneous circulation by iontophoresis were expressed as percentage change from that prior to iontophoresis in the resting condition (averaged over 20 seconds and set at 0 %). For responses to ACh, average skin blood flow was calculated over the final 20 seconds of the interval between successive pulses and between 40 to 60 seconds after the final pulse. For SNP, average skin blood flow was calculated over the final 30 seconds of the interval between successive pulses and at 90 to 120 seconds after the final pulse. Responses to NA are shorter-lasting (Hendry and Marshall 2004) therefore the minimum skin blood flow was identified between each pulse and between 0 to 60 seconds after the final pulse.

Statistical analyses were conducted using IBM SPSS for Windows version 20 (IBM SPSS Statistics, USA). An α value of 0.05 was used to determine statistical significance. An independent samples *t*-test was utilised to compare participant characteristics and blood pressures between ethnic groups. A paired samples *t*-test was utilised to assess within-group change in blood pressure from pre to post iontophoresis. The skin blood flow data were not normally distributed therefore group comparisons were conducted utilising a Mann-Whitney *U* test. Half-maximal effective dose (ED50) expressed as 95 %

confidence intervals was calculated using GraphPad (Version 5, USA). Maximal skin blood flow (for ACh and SNP) and minimum skin blood flow (for NA) was calculated for each participant and compared between ethnic groups. The point at which the skin blood flow was at a maximum or minimum point was not always identified following the final pulse, therefore maximum skin blood flow was taken from wherever it was highest and minimum skin blood flow was taken from whenever it was lowest. Parametric data in text are presented as mean (SD). Non-parametric data are presented as median (interquartile range - IQR). Data displayed in figures are presented as mean (SD). Effect sizes, where appropriate, were calculated using Cohen's d for parametric data (denoted by *d* in text) and Rosenthal's *r* for non-parametric data (denoted by *r* in text).

RESULTS

Both ethnic groups were of similar age (CAU: 21[3] years, AFD: 21[2] years), height (CAU: 1.8[0.1] m, AFD: 1.8[0.1] m) and mass (CAU: 73.4[10.7] kg, AFD: 73.6[12.2] kg). Blood pressure did not differ between ethnic groups prior to iontophoresis at each skin site (average over 5 recordings) for ACh, SNP and NA (Table 1). Blood pressure did not differ between ethnic groups post iontophoresis, or within group's pre to post iontophoresis.

[Insert Table 1 here]

Prior to iontophoresis local skin blood flow (Table 2) and skin temperature (Table 3) did not differ between ethnic groups at any skin site except that AFD had a lower resting skin blood flow at the glabrous toe compared to CAU prior to SNP application ($P = 0.014$) however this did not translate to a difference in skin temperature (Table 3). As expected, resting skin blood flow was higher in the glabrous skin regions compared to the non-glabrous regions and this was more marked in the fingers (Table 2). Not all participants could receive the first pulse of iontophoresis due to high skin resistance; therefore only those who

successfully received the first pulse were included in the analyses (Tables 2 and 3).

[Insert Table 2 here]

[Insert Table 3 here]

Skin blood flow responses to acetylcholine (ACh)

Dose response curves to ACh were achieved in the non-glabrous regions (finger, toe and forearm; Fig. 1a, 1c and 1e respectively) but not the glabrous regions (finger and toe pads) where the skin blood flow response remained unchanged (Fig. 1b and 1d respectively). In the non-glabrous finger, AFD demonstrated less vasodilatation for a given current (Fig. 1a, P = 0.043 - 0.014, r = 0.48 - 0.52), a lower maximum vasodilatation (Mdn [IQR], AFD n = 11, 41[234] %, CAU n = 12, 351[451] %, P = 0.011, r = 0.53) and a greater ED50 (95% confidence intervals, AFD = 136 µA – 223 µA, CAU = 40 µA – 117 µA, P < 0.001) compared with CAU. In the non-glabrous toe, again AFD demonstrated less vasodilatation for a given current (Fig. 1c, P = 0.024 - 0.023, r = 0.50 - 0.68) and a lower maximum vasodilatation (Mdn [IQR], AFD n = 9, 116[318] %, CAU n = 12, 484[720] %, P = 0.018, r = 0.51) compared with CAU, however ED50 was similar. There were no skin blood flow differences between groups for the forearm (Fig. 1e).

[Insert Fig. 1 here]

Skin blood flow responses to sodium nitroprusside (SNP)

Dose response curves to SNP were observed in the forearm skin site for both groups (Fig. 2e). Dose response curves were also obtained for the non-glabrous finger and toe for CAU but not AFD (Fig. 2a and 2c). No dose response curves were achieved in the glabrous sites (finger and toe) for CAU or

AFD (Fig. 2b and 2d). In the forearm skin site AFD demonstrated less vasodilatation for a given current (Fig. 2e, P = 0.027 - 0.004, r = 0.46 - 0.58) and a greater ED50 (95% confidence intervals, AFD = 130 μA – 167 μA, CAU = 80 μA – 107 μA, P < 0.001) compared with CAU. Following the final pulse on the glabrous toe skin site AFD had a smaller skin blood flow response compared with CAU (P = 0.018, r = 0.61).

[Insert Fig. 2 here]

Skin blood flow responses to noradrenaline (NA)

Vasoconstriction in response to NA was achieved in the forearm, non-glabrous finger and glabrous toe. Vasoconstriction was also achieved in the glabrous finger and non-glabrous toe of AFD but not CAU; however there were no skin blood flow differences at any skin site between ethnic groups. There were no ethnic differences for minimum skin blood flow for any skin site (Table 4).

[Insert Table 4 here]

The responses to transdermal delivery of ACh, SNP and NA to the various skin regions in the two ethnic groups are summarised in Table 5.

[Insert Table 5 here]

DISCUSSION

In the present study, comparing young male CAU and AFD participants, we observed a lower increase in skin blood flow for a given current in response to ACh in the non-glabrous finger and non-glabrous toe in AFD (Fig. 1a and 1c, respectively); however these differences were not repeated with SNP (Fig. 2). Furthermore, there were no differences between ethnic groups in response to NA at any skin site. These findings allow us to partly accept our hypothesis

regarding ACh and SNP whilst we reject our hypothesis for NA. This is in contrast to other studies which have investigated whole arm or systemic responses between ethnic groups (Jones et al. 1999; Cardillo et al. 1999; Rosenbaum et al. 2002). We observed a lower endothelium-dependent vasodilatation (Fig. 1) in AFD than CAU in the non-glabrous finger and non-glabrous toe skin sites although responses at the forearm did not differ between groups. The non-glabrous toe skin blood flow responses for CAU displays an initial increase in skin blood flow, followed by a decrease and a subsequent increase. Looking at the individual data it appears that this pattern is primarily driven by two CAU who had an early exaggerated response to ACh where as all other CAU demonstrated a linear increase in skin blood flow. We are unsure why two CAU demonstrated such an early exaggerated response to ACh. Although we observed a significantly lower skin blood flow response in the non-glabrous toe skin site between CAU and AFD following the final pulse, only three AFD were remaining at that data point thus caution should be exercised when considering this time point.

Our results from the forearm skin site, displaying similar skin blood flow responses, appear to support some previous findings (Kahn et al. 2002) but not all (Jones et al. 1999; Cardillo et al. 1999). In the present study, whilst the forearm microcirculation appears to respond similarly in CAU and AFD to iontophoresis of ACh, it seems that the control of the microcirculation of the fingers and toes in the non-glabrous skin sites differs between CAU and AFD; this may play a role in the increased susceptibility of AFD to cold injuries.

The present study supports the findings that a difference exists in response to SNP at the forearm (Stein et al. 1997; Cardillo et al. 1998; Cardillo et al. 1999; Gainer et al. 2001; Rosenbaum et al. 2002). Reduced vasodilatation in AFD compared with CAU in response to SNP would suggest an altered function at the vascular smooth muscle cells. However, an electric-induced hyperaemic response (galvanic response) is prominent at the cathode site where SNP is used (Morris and Shore 1996). The increase in skin blood flow observed upon

electrical stimulation has been shown to recruit mechano-insensitive C-units (Schmelz et al. 2000) which release vasodilators calcitonin gene related peptide with smaller contributions from substance P (Sauerstein et al. 2000). We attempted to attenuate this response by using a low current (i.e. 25 μA) in short durations (i.e. 20 seconds). During pilot testing we observed an increase in skin blood flow (~2100 % increase from rest following the final pulse, *n* = 5) in the forearm when water for injection was used at the cathode whereas the other skin sites did not demonstrate any significant increase in skin blood flow. This is therefore a limitation of the iontophoresis technique in that the responses to iontophoresis of SNP in the forearm circulation may, in part, be influenced by a sensory component / galvanic response (Morris and Shore 1996), but the responses to SNP in other skin sites appear to be nitric oxide driven. Responses to SNP at other sites did not differ between groups except following the final pulse on the glabrous toe (Fig. 2d). We are unsure why this difference between groups occurred but feel that we cannot comment on the possible physiological implications of this due to the low number of participants remaining in the AFD group (*n* = 3) at this time / current application.

It was expected that NA would cause a greater decrease in skin blood flow in AFD compared with CAU; this was not the case in the present study. Previous research has showed that CAU and AFD do not appear to differ in response to intra-arterial infusion of angiotensin II (Jones et al. 1999); others have shown that intra-arterial infusion of phenylephrine causes a greater vasoconstrictor response in AFD than CAU (Stein et al. 2000). Both vasoactive agents appear to exert their vasoconstrictor effects by modulating intracellular calcium levels but the reason for the discrepancy between studies may be due to different mechanism of action; angiotensin II acts on the endothelium as well as the smooth muscle cell, whereas phenylephrine, an α_1 receptor agonist, acts upon the smooth muscle cell (Pueyo and Michel 1997; Rang et al. 2012). NA is able to activate α (vasoconstrictor) as well as β (vasodilatory) adrenergic receptors (Bylund et al. 1994; Rang et al. 2012) which may mask an increased sensitivity in α receptors in AFD as previously reported (Stein et al. 2000). Human

vascular smooth muscle contains several types of α receptors with the α_{2C} subtype appearing to become active under conditions such as skin cooling (Chotani et al. 2004). In support of this, previous studies have shown vasoconstriction responses to local cooling is governed primarily by α_2 receptors (Ekenvall et al. 1988), more specifically α_{2C} subtype (Chotani et al. 2000). These specific receptors are translocated from the Golgi to the vascular smooth muscle cell surface facilitated by Rho kinase (Bailey et al. 2004; Honda et al. 2007). During forearm skin cooling, cutaneous vascular conductance is significantly attenuated following Rho kinase inhibition which may be as a result of a reduced translocation of α_{2C} receptors to the vascular smooth muscle cell surface (Thompson-Torgerson et al. 2007). We previously observed a lower skin blood flow in AFD during hand cooling compared with CAU (Maley et al. 2014); whether there are ethnic differences in α_{2C} receptor subtype is not known.

Whilst the present study provides evidence of an attenuated response to ACh in AFD, the precise mechanism(s) controlling this response is not known. ACh binds to muscarinic receptors on the endothelial surface and produces mediators to effect vasodilatation (Furchgott 1983; Komori and Suzuki 1987; Shore 1996; Shibasaki et al. 2002). These mediators include prostanoids, produced by the cyclooxygenase (COX) enzyme, which are subsequently metabolised to various prostaglandins, including prostacyclin, a known vasodilator (Duffy et al. 1998; Parkington et al. 2004; Félétou 2011). Previous investigations have demonstrated that responses to ACh in the forearm circulation are predominantly mediated through prostanoid-dependent pathways, and an attenuated vasodilatation is observed in the forearm circulation with COX inhibition compared with a control (Noon et al. 1998; Holowatz et al. 2005). However, there are further increases in non-glabrous finger skin blood flow in response to ACh when COX is inhibited in young healthy males (Hendry and Marshall 2004). Thus, it appears that there may be a greater contribution of prostanoid vasoconstrictor products in the finger compared with the forearm. From this, it may be that the balance between

vasoconstrictor and vasodilator COX products may differ between groups. Even though responses to SNP, a nitric oxide donor, is similar between groups this does not rule out possible differences in nitric oxide bioavailability affecting ACh induced vasodilatation. Evidence of a reduced nitric oxide bioavailability in AFD compared with CAU has been previously reported and is attributable to an increased superoxide production (Kalinowski et al. 2004; Mata-Greenwood and Chen 2008; Feairheller et al. 2011); this type of oxidative stress and diminished nitric oxide bioavailability has been attributed to vascular disorders such as hypertension (Griendling and FitzGerald 2003) as well as a reduced nitric oxide-dependent vasodilatation in aged skin (Holowatz and Kenney 2010).

Although the present study examined skin blood flow responses to vasoactive agents in individuals at rest at environmental temperature of 23 °C to 24 °C, we have previously reported that AFD experience an attenuated vasodilatation compared with CAU during rewarming following hand cooling (Maley et al. 2014). Low perfusion of tissue during exposure to cold and upon rewarming can be a factor leading to environmental injuries such as NFCI (Endrich et al. 1982; Jia and Pollock 1997; Jia and Pollock 1998). From the present study, as NA responses were similar between ethnic groups it is suggested that the skin blood flow differences observed during the previous study may, in part, be due to differences in the reactivity of the endothelium in AFD compared with CAU which may help explain why this group is more susceptible to freezing and non-freezing cold injuries. The precise mechanism underpinning differences in rewarming times following local cold exposure between AFD and CAU such as that seen previously (Maley et al., 2014) has not yet been fully elucidated. Clues come from findings such as those of Hope et al. (2014) who reported that the nitric oxide donor glyceryl trinitrate improves rewarming times following foot cooling in individuals with cold sensitivity (sub-clinical NFCI). However, we did not observe any differences in skin temperature between uninjured AFD and CAU to the same foot cooling protocol (Maley et al. 2014) therefore, in support of the present study, the role of nitric oxide in CAU and AFD appears similar in response to short duration (2 minute) foot cooling and subsequent rewarming.

It is concluded that young healthy AFD have an attenuated endothelium-dependent vasodilatation, compared with CAU, in non-glabrous sites of the fingers and toes. This may help explain why AFD are more susceptible to cold injuries which occur predominantly in the peripheries.

ACKNOWLEDGEMENTS

The authors wish to thank the participants for volunteering for the study.

ETHICAL STANDARDS

This study complied with The Declaration of Helsinki, as adopted at the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, 1964 and last amended at the 64th World Medical Association General Assembly, Brazil 2013. This study complied with the Council of Europe (2005). Additional Protocol to the convention on human rights and biomedicine concerning biomedical research. European Treaty Series No. 195, Strasbourg 25 January 2005. Additionally, the study received ethical and scientific approval from the Science Faculty Ethics Committee, prior to recruitment of volunteer participants, who gave informed written consent.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

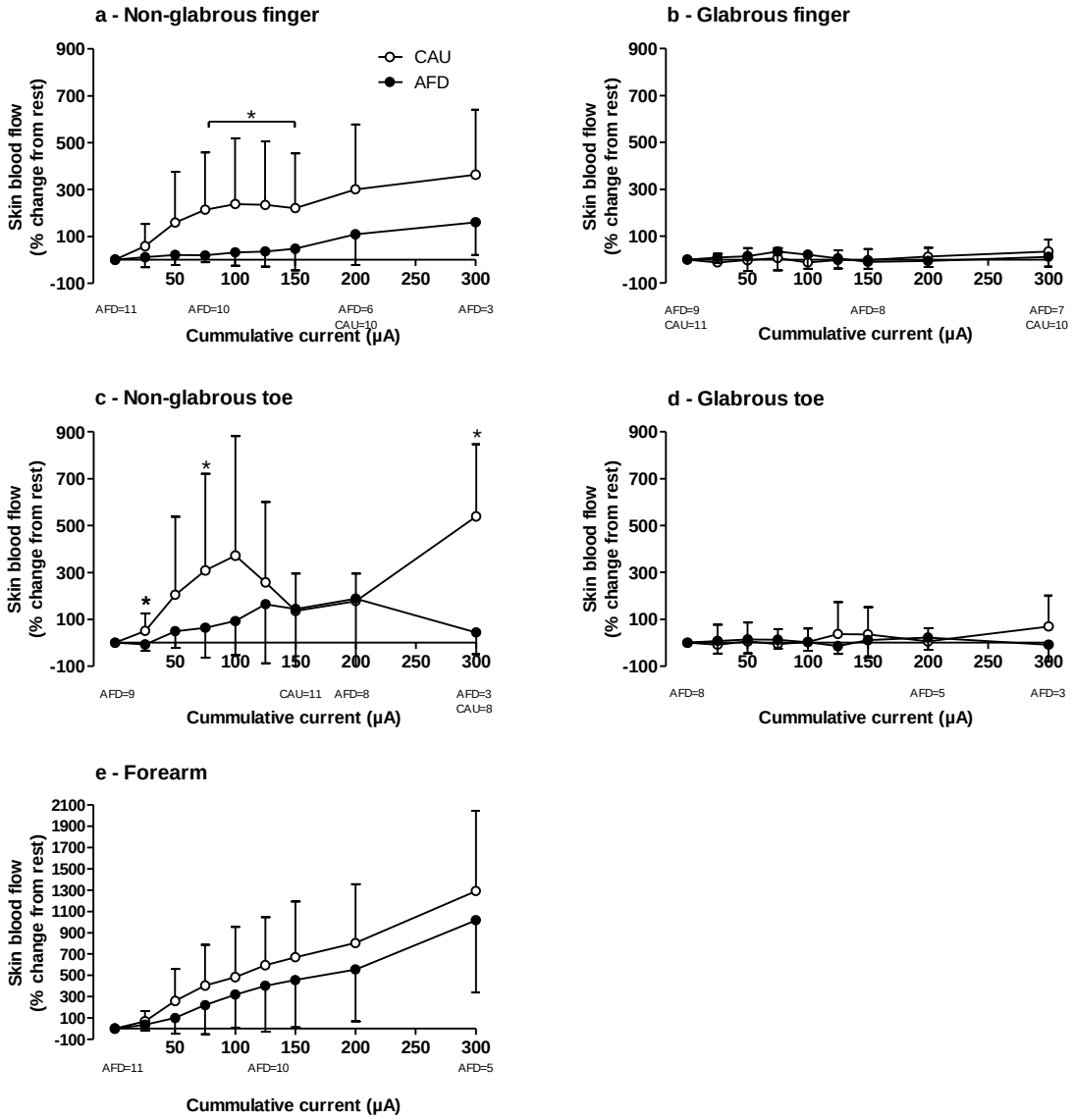

Fig. 1 Mean (SD) skin blood flow responses to iontophoresis of acetylcholine in the non-glabrous finger (a), glabrous finger (b), non-glabrous toe (c) and glabrous toe (d) and forearm (e). n = 12, unless stated. *Significant difference between CAU and AFD, $P < 0.05$. Note scale change for Fig 1e.

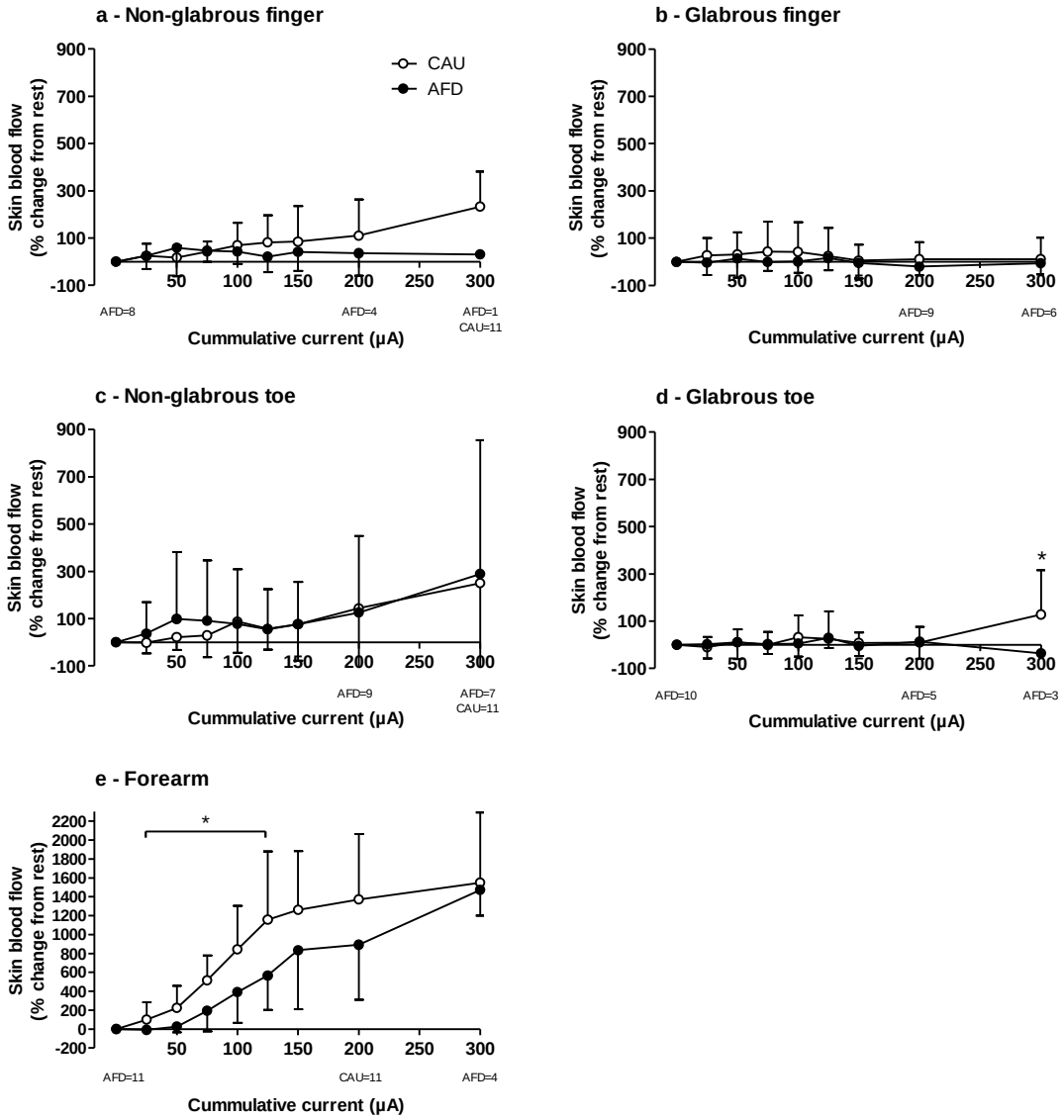

Fig. 2 Mean (SD) skin blood flow responses to iontophoresis of sodium nitroprusside in the non-glabrous finger (a), glabrous finger (b), non-glabrous toe (c) and glabrous toe (d) and forearm (e). n = 12, unless stated. *Significant difference between CAU and AFD, $P < 0.05$. Note scale change for Fig 2e.

Table 1 Mean (SD) blood pressure prior to iontophoresis (*n* = 24)

	Blood Pressure (mmHg)					
	Acetylcholine		Sodium nitroprusside		Noradrenaline	
Variable	CAU	AFD	CAU	AFD	CAU	AFD
Systolic	120(11)	121(9)	119(9)	124(9)	120(7)	123(9)
Diastolic	60(6)	64(8)	62(5)	65(8)	62(7)	63(5)
MAP	80(6)	83(8)	81(5)	85(8)	81(6)	83(6)

MAP = Mean Arterial Pressure.

Table 2 Median (IQR) local skin blood flow (laser Doppler units) prior to iontophoresis at each skin site

	Laser Doppler Units					
	Acetylcholine		Sodium nitroprusside		Noradrenaline	
Site	CAU	AFD	CAU	AFD	CAU	AFD
Forearm	15(10)	18(16)	16(8)	15(6)	17(17)	11(14)
	n=12	*n*=11	*n*=12	*n*=11	*n*=11	*n*=11
NGF	58(24)	53(21)	61(31)	36(117)	55(35)	59(102)
	n=12	*n*=11	*n*=12	*n*=8	*n*=12	*n*=11
GF	399(182)	326(370)	341(249)	255(293)	247(283)	122(462)
	n=11	*n*=9	*n*=12	*n*=12	*n*=11	*n*=11
NGT	18(37)	11(34)	20(22)	11(8)	11 (27)	16(18)
	n=12	*n*=9	*n*=12	*n*=12	*n*=12	*n*=11
GT	38(124)	13(53)	44(61)*	15(24)	71(163)	41(94)
	n=12	*n*=8	*n*=12	*n*=10	*n*=11	*n*=9

*Significant difference between groups ($P < 0.05$). NGF = Non-glabrous finger, GF = Glabrous finger, NGT = Non-glabrous toe, GT = Glabrous toe.

Table 3 Median (IQR) local skin temperature prior to iontophoresis at each skin site

	Skin Temperature (°C)					
	Acetylcholine		Sodium nitroprusside		Noradrenaline	
Site	CAU	AFD	CAU	AFD	CAU	AFD
Forearm	28.9(1.2)	28.4(1.1)	28.8(0.6)	27.7(1.6)	29.4(1.9)	29.2(1.4)
	n=12	*n*=11	*n*=12	*n*=11	*n*=11	*n*=11
NGF	29.1(1.9)	28.7(1.2)	29.6(2.7)	27.3(2.0)	30.2(0.8)	29.5(2.4)
	n=12	*n*=11	*n*=12	*n*=8	*n*=12	*n*=11
GF	29.7(2.0)	30.5(4.8)	30.2(3.6)	28.8(4.8)	30.4(1.9)	30.2(3.3)
	n=11	*n*=9	*n*=12	*n*=12	*n*=11	*n*=11
NGT	26.6(3.3)	27.1(3.2)	26.6(2.4)	27.0(3.5)	27.6(3.4)	27.6(3.4)
	n=12	*n*=9	*n*=12	*n*=12	*n*=12	*n*=11
GT	26.3(3.9)	25.5(2.7)	25.4(4.4)	24.3(2.9)	28.2(2.4)	26.9(3.4)
	n=12	*n*=8	*n*=12	*n*=10	*n*=11	*n*=9

NGF = Non-glabrous finger, GF = Glabrous finger, NGT = Non-glabrous toe, GT = Glabrous toe.

Table 4 Median (IQR) and mean (SD) maximum percentage change in skin blood flow in response to iontophoresis of noradrenaline

	Ethnicity	
Site	CAU	AFD
Forearm ^	-39[15] % *n* = 11	-29[55] % *n* = 11
Non-glabrous finger	-65[23] % *n* = 12	-54[25] % *n* = 11
Glabrous finger	-64[21] % *n* = 11	-65[25] % *n* = 11
Non-glabrous toe	-44[32] % *n* = 12	-46[27] % *n* = 11
Glabrous toe	-58[27] % *n* = 11	-46[33] % *n* = 9

^ Median (IQR).

Table 5 Summary of skin blood flow results

Group	Response to Acetylcholine	Response to Sodium nitroprusside	Response to Noradrenaline
CAU	↑ forearm, NGF, NGT	↑ forearm, NGF, NGT	↓ forearm, NGF, GT
AFD	↑ forearm, NGF, NGT	↑ forearm	↓ forearm, NGF, GF, NGT, GT
CAU *vs.* AFD	NGF and NGT CAU > AFD	Forearm CAU > AFD	No differences

NGF = Non-glabrous finger, GF = Glabrous finger, NGT = Non-glabrous toe, GT = Glabrous toe.

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